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TITLE: Alterations in whole-body insulin sensitivity resulting from repeated eccentric exercise of a single muscle group: a pilot investigation

SHORT RUNNING TITLE: Muscle damage and insulin resistance

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Abstract

Unaccustomed eccentric exercise using large muscle groups elicits soreness, decrements in physical function and impairs markers of whole-body insulin sensitivity; although these effects are attenuated with a repeated exposure. Eccentric exercise of a small muscle group (elbow flexors) displays similar soreness and damage profiles in response to repeated exposure. However, it is unknown whether damage to small muscle groups impacts upon whole-body insulin sensitivity. This pilot investigation aimed to characterize whole-body insulin sensitivity in response to repeated bouts of eccentric exercise of the elbow flexors. Nine healthy males completed two bouts of eccentric exercise separated by 2 weeks. Insulin resistance (updated homeostasis model of insulin resistance, HOMA2-IR) and muscle damage profiles (soreness and physical function) were assessed before, and 48 h after exercise. Matsuda insulin sensitivity indices (ISI_{Matsuda}) were also determined in 6 participants at the same time points as HOMA2-IR. Soreness was elevated, and physical function impaired, by both bouts of exercise (both $P < 0.05$) but to a lesser extent following bout 2 (time x bout interaction, $P < 0.05$). Eccentric exercise decreased ISI_{Matsuda} after the first but not the second bout of eccentric exercise (time x bout interaction $P < 0.05$). Eccentric exercise performed with an isolated upper limb impairs whole-body insulin sensitivity after the first, but not the second, bout.

Keywords: glucose; glycemia; insulin resistance; metabolic control; muscle damage repeated bout.

Abbreviations

GLUT-4: glucose transporter isoform 4

HOMA2-IR: updated homeostasis model of insulin resistance

- 50 iAUC: incremental area under the curve
- 51 ISI_{Matsuda} : Matsuda insulin sensitivity index
- 52 MVC: maximal voluntary contraction
- 53 OGTT: oral glucose tolerance test
- 54
- 55
- 56

Introduction

Insulin sensitivity indices predict the risk of developing metabolism-related diseases i.e. type 2 diabetes and cardiovascular disease (The DECODE Study Group & The European Diabetes Epidemiology Group, 1999; Zavaroni et al., 1989), even when only the “healthy” range of indices are considered (Ning et al., 2012). Accordingly, understanding how insulin sensitivity responds to stimuli can give insight into metabolic disease risk in currently healthy populations. Whilst regular exercise alongside lifestyle interventions can prevent metabolic disease (Knowler et al., 2002), the acute effects of exercise on whole-body glucose metabolism are equivocal. Following a single bout of exercise, glucose tolerance has been shown to improve (Bonen, Ball-Burnett, & Russel, 1998), deteriorate, or remain stable (Gonzalez, Veasey, Rumbold, & Stevenson, 2013), relative to rest. Numerous factors are postulated to explain these discrepancies (including metabolic and nutritional status of the population, modality, volume and intensity of exercise), one of which is muscle damage induced by exercise with an eccentric component, and associated impairment of insulin sensitivity (Gonzalez, 2014).

Typically, the exercise paradigms employed to study muscle damage involve large muscle groups or whole-body exercise, i.e., downhill running (Cook, Myers, Kelly, & Willems, 2014; Green et al., 2010), or eccentric exercise of knee flexors (Paschalis et al., 2011). These models produce acute metabolic alterations indicative of reduced insulin sensitivity when measured at 48 h (Green et al., 2010; Paschalis et al., 2011) post-exercise. This effect is only present when exercise is unaccustomed, and is abolished or reversed with multiple bouts (Green et al., 2010; Paschalis et al., 2011). For damaging exercise of a small muscle group, similar profiles of damage, recovery and protection on repeated-bouts have been observed (Howatson, van Someren, & Hortobagyi, 2007), but the effect of damaging exercise of a small muscle group on whole-body insulin sensitivity is unknown. If whole-

body insulin sensitivity can be modified by acute exercise of small muscle groups, such as the elbow flexors of a single limb (constituting <6% of total lean mass (Araujo et al., 2010)), this could reveal an avenue to explore potentially beneficial adaptations with multiple bouts, which may have implications during forced inactivity or immobilization.

Accordingly, this pilot investigation aimed to assess whole-body insulin sensitivity during an oral glucose tolerance test (OGTT), in response to two bouts of eccentric exercise of the elbow flexors, separated by 14 days. We hypothesized that damaging exercise of a single muscle group would impair whole-body insulin sensitivity after the first, but not the second bout.

Materials and methods

Participants

Six male participants completed the full protocol, whilst a further three males provided fasting samples only. Thus, postprandial OGTT data are $n = 6$ whilst all other data are $n = 9$ (participant characteristics are presented in Table 1). All participants were naïve to regular resistance exercise.

Study design

Participants visited the laboratory on 6 occasions; twice to complete the eccentric exercise protocol (separated by 2 weeks), and 4 times for blood sampling in line with assessment of physical function and soreness (muscle damage markers). Blood sampling (including OGTT) and damage marker assessments were performed prior to, and 48 h following damaging exercise. The eccentric exercise protocol was performed on an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems Inc. NY, USA) and comprised 8×5 maximal eccentric contractions of the left elbow flexors at 30°d.s^{-1} ; each set separated by 90 s rest.

107

108 *Subjective soreness and physical function*

109 Subjective soreness was determined using 200 mm visual analogue scales during full range of
110 movement of the elbow flexors. Physical function was taken as the peak value attained during
111 3 isometric maximal voluntary contractions (MVC) of the elbow flexors, each performed
112 with 90° flexion of the elbow, separated by 120 s rest and following a standardized warm-up.

113

114 *OGTT and blood sampling*

115 Participants were asked to maintain a similar carbohydrate intake throughout to minimize
116 effects of diet on insulin sensitivity. Blood sampling was always performed after a 12-h fast.
117 Participants were instructed to eat their evening meal prior to trials at a standardized time, to
118 eat the same meal before all trials, and to refrain from exercise for 24 h prior to blood
119 sampling in accordance with standardization for postprandial glycemia testing guidelines
120 (Brouns et al., 2005). For those who undertook the OGTT, 75 g of glucose (82 g dextrose
121 monohydrate, corrected for moisture; Myprotein, Cheshire, UK) was dissolved in 300 ml of
122 water and ingested within 5 min. Finger-prick blood samples were taken before (0 min), and
123 15, 30, 45, 60, 90 and 120 min following ingestion, and analyzed immediately for blood
124 glucose concentration (Biosen C_line, EKF Diagnostics, Magdeberg, Germany), whilst a 250
125 µL EDTA-microvette was filled with whole blood, before centrifugation (10 min at 3000
126 rpm). By revisiting glucose data obtained in duplicate from one of our previous studies
127 (Gonzalez & Stevenson, 2012), we are able to report reliability statistics, which include the
128 combined variability of sample collection and analysis. Across 196 pairs of samples (range
129 3.60-8.81 mmol/L), the standardized typical error was 0.12 mmol/L (95%CI: 0.11, 0.13
130 mmol/L) and the coefficient of variation was 1.7%. Plasma was stored at -80°C for

subsequent determination of insulin concentrations by commercially available ELISA (IBL International GmbH, Hamburg, Germany; intra-assay coefficient of variation: 6%).

Calculations and statistics

Insulin sensitivity was estimated in the fasted state, using the updated homeostasis model of insulin resistance (HOMA2-IR; reciprocal of insulin sensitivity (Levy, Matthews, & Hermans, 1998)) and in the postprandial state (OGTT), using the Matsuda insulin sensitivity index ($ISI_{Matsuda}$ (Matsuda & DeFronzo, 1999)). Postprandial glucose and insulin concentrations were converted into time-averaged incremental areas under the curve (iAUC) as has been previously used (Gill et al., 2004). All analyses were performed using Prism v6 (Graphpad Software, San Diego, CA). Data were checked for normal distribution (D'Agostino & Pearson omnibus normality test) and log transformed if appropriate, prior to analysis. The difference in work done between bout 1 and bout 2 was assessed by a paired samples t-test. Two-way [time (pre vs. post) x bout (bout 1 vs. bout 2)] repeated measures ANOVA were used to examine differences in fasting blood variables, OGTT data, MVC and soreness ratings. Data are presented as means \pm SEM unless stated otherwise, and statistical significance was set at $P < 0.05$.

Results

Total work done during eccentric exercise was similar between bout 1 (2501 ± 205 kJ) and bout 2 (2527 ± 215 kJ; $P = 0.738$). Eccentric exercise elicited increases in soreness on both bouts ($P = 0.003$). Soreness was lower on the second bout vs. the first ($P = 0.001$) and significantly attenuated (time \times bout interaction $P = 0.001$; Figure 1A). MVC decreased after both bouts (main effect of time, $P < 0.001$). No significant main effect of bout was detected

($P = 0.218$), but the reduction in MVC post-damaging exercise was attenuated on repeated bouts (time \times bout interaction, $P = 0.019$; Figure 1B).

Fasting indices of insulin sensitivity (glucose and insulin concentrations, and HOMA2-IR) were unaffected by the intervention and neither was the glucose nor insulin iAUC (Table 2 and Figure 2). ISI_{Matsuda} did not display significant main effects for time or bout (both $P > 0.05$) but the reduction in ISI_{Matsuda} observed after bout 1 was abolished after bout 2 (time \times bout interaction, $P = 0.030$, Figure 1C) indicating preserved insulin sensitivity after the second bout.

Discussion

These data indicate that: 1) unaccustomed eccentric exercise of a single upper-body limb reduces insulin sensitivity at the whole-body level, detectable in the postprandial state; 2) the impairment in insulin sensitivity is abolished following a second bout of damaging eccentric exercise.

Previous work has demonstrated acute reductions in insulin sensitivity following downhill running are absent following a second bout (Green et al., 2010), and others have shown that after 8 bouts, eccentric exercise of the knee flexors *increases* fasting insulin sensitivity indices (Paschalis et al., 2011). Here we demonstrate that a single exposure to eccentric exercise of a single, small muscle group (left elbow flexors) induces an adaptive response, whereby full protection from acute impairment of insulin sensitivity is observed. Whether eccentric exercise of an upper limb has the capacity to positively influence insulin sensitivity over a longer time-course however, warrants further investigation. If this is the case, then one can envisage potential application during imposed inactivity or immobilization of lower limbs.

It has been suggested that due to relatively low insulin concentrations used to calculate HOMA2-IR (fasting vs. a clamp procedure or postprandial), this measure represents a different balance of sensitivity (hepatic vs. peripheral) than the $ISI_{Matsuda}$ (Matsuda & DeFronzo, 1999; Radziuk, 2014). Accordingly, the reduction in $ISI_{Matsuda}$ seen in the present study, when viewed in light of the lack of change in HOMA2-IR, suggests that eccentric exercise reduced peripheral (but not hepatic) insulin sensitivity.

Numerous mechanisms have been proposed to underlie muscle damage-induced reductions in insulin sensitivity. These include, a decrease in glucose transporter isoform 4 (GLUT-4) at the plasma membrane due to reduced GLUT-4 transcription and thus GLUT-4 protein content (Kristiansen, Jones, Handberg, Dohm, & Richter, 1997), associated with reduced muscle glucose transport manifest under hyperinsulinaemia but, intriguingly, elevated glucose transport when not exposed to insulin (Asp & Richter, 1996). This provides another potential explanation for the detectable reductions in $ISI_{Matsuda}$ but not in HOMA2-IR. Secondly, systemic factors released by damaged muscle including cytokines such as tumor necrosis factor- α may also be implicated an impaired ability of insulin to stimulate insulin receptor substrate-1, phosphatidylinositol 3-kinase and Akt (protein kinase B) (Asp, Dagaard, Kristiansen, Kiens, & Richter, 1996; Del Aguila et al., 2000; Krogh-Madsen, Plomgaard, Moller, Mittendorfer, & Pedersen, 2006; Liao, Zhou, Ji, & Zhang, 2010). Whilst our data are unable to give insight into which of these mechanisms is responsible, given the relatively small muscle group used (<6% of total lean mass (Araujo et al., 2010)), the impact at the systemic level is noteworthy. This indicates that, either a very small decrease in total GLUT-4 content has implications for insulin sensitivity at the whole body level, and/or damage to small muscle groups produces adequate release of systemic factors (*ie.* cytokines) to impair the action of a sufficient mass of insulin sensitive tissue to influence whole-body metabolism.

In conclusion, these data indicate that eccentric exercise of a single upper limb, inhibits whole-body insulin sensitivity 48 h after the first bout, and such a reduction is not apparent after a second bout.

Novelty statement: Eccentric exercise of large muscle groups (leg flexors of both legs, or whole-body exercise) is known to impair whole-body insulin sensitivity after an initial exposure, with protection from this effect demonstrated with subsequent bouts. This is the first study to demonstrate that eccentric exercise with a single small muscle group (elbow flexors of a single arm) impairs insulin sensitivity following the first bout, but not following a second bout.

Practical application statement: In developing strategies to modulate insulin sensitivity, activating large muscle groups may not necessarily be required to elicit a response at the whole-body level. Eccentric exercise using upper limbs is likely sufficient to influence whole-body insulin sensitivity and this pilot work highlights a new strategy to potentially influence metabolism.

Author contributions

All authors contributed to study design, data collection and analysis, drafting, editing and approved the final article.

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Conflict of interest

229 The authors declare no conflict of interest.

230

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Figure legends

Figure 1. Subjective soreness (A), maximal voluntary force production (B) and insulin sensitivity indices (C) before and 48 h after 2 bouts of eccentric exercise using the elbow flexors of an upper limb in males. MVC, maximum voluntary contraction force; $ISI_{Matsuda}$, Matsuda insulin sensitivity index (Matsuda & DeFronzo, 1999). Data expressed as means \pm SEM. *, significant main effect of time; #, significant main effect of bout; ^, significant time x bout interaction effect ($P < 0.05$).

Figure 2. Blood glucose (A, B) and plasma insulin (C, D) concentrations during an OGTT prior to and 48 h following, an initial (A, C) and second (B, D) bout of eccentric exercise using the elbow flexors of an upper limb in males. Data expressed as means \pm SEM.

List of tables

Table 1. Participant characteristics.

Variable	Fasting Data ¹		OGTT Data ²		Independent t-test (<i>P</i>)
	Mean ± SEM	Range	Mean ± SEM	Range	
Age (y)	21 ± 1	19 – 26	21 ± 1	19 – 26	0.749
Stature (cm)	180 ± 2	173 – 188	181 ± 2	173 – 186	0.845
Body mass (kg)	76.9 ± 2.8	65 – 89.2	77.0 ± 3.1	68.7 – 86.2	0.997
Body mass index (kg/m ²)	23.6 ± 0.6	19.9 – 26.0	23.5 ± 0.8	19.9 – 26.0	0.923

¹, *n* = 9; ², *n* = 6.

Table 2. Indices of insulin sensitivity in response to acute and repeated exposure to eccentric exercise.

Variable	Bout 1		Bout 2		ANOVA
	Pre	48 h post	Pre	48 h post	time x bout interaction (<i>P</i>)
Fasting glucose ¹ (mmol/L)	4.45 ± 0.13	4.46 ± 0.20	4.46 ± 0.14	4.57 ± 0.18	0.756
Fasting insulin ¹ (pmol/L)	128 ± 36	149 ± 54	147 ± 40	160 ± 46	0.910
HOMA2-IR ¹	2.25 ± 0.62	2.60 ± 0.92	2.54 ± 0.65	2.75 ± 0.73	0.874
Glucose iAUC ² (mmol/L)	1.47 ± 0.23	1.29 ± 0.19	1.91 ± 0.22	1.27 ± 0.33	0.285
Insulin iAUC ² (pmol/L)	139 ± 36	153 ± 26	158 ± 17	144 ± 38	0.160

Data expressed as means ± SEM. ¹, *n* = 9; ², *n* = 6; HOMA2-IR, updated homeostasis model of insulin resistance (Levy et al., 1998); iAUC, incremental time-averaged area under the curve.